QUANTITATIVE *IN VIVO* MAGNETIC RESONANCE IMAGING OF SPIO-LABELED CELLS IN A CERVICAL CANCER MOUSE MODEL

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INTRODUCTION

- Understanding the role of immune cells is *in vivo* is important for a number of disease processes, particularly with cancer and immunotherapies.
- Magnetic resonance imaging (MRI) can be used to monitor immune cells *in vivo* by labeling with a superparamagnetic iron oxide (SPIO) contrast agent.
- These methods allow us to detect immune cell recruitment and migration patterns by acquiring MRIs and analyzing changes in image contrast within a region of interest.
- Traditionally, we have used a bSSFP MRI pulse sequence to perform studies evaluating SPIO labeled components[1]. Unfortunately, this sequence lacks *specificity* in detecting the contrast effects of SPIO. Labeled cells can be detected by an increase in negative (dark) contrast, but necrosis results in similar contrast. This was confirmed using simultaneous positron emission tomography (PET)/MRI (Fig. 1).
- Additionally, the previous method does not support true quantitative evaluation of cellular recruitment.
- We propose use of a second sequence, TurboSPI[2], that has greater *specificity* and supports cellular quantification.

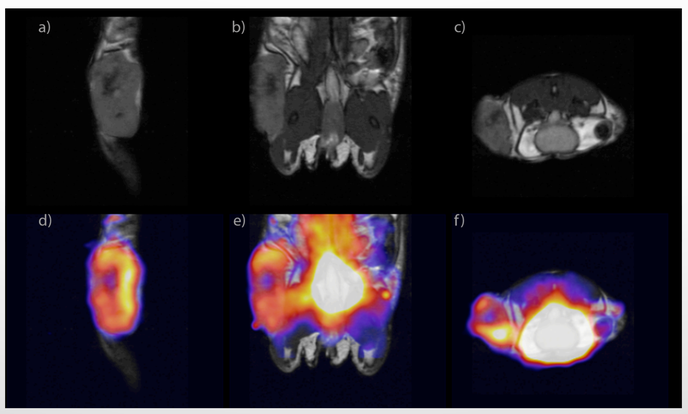

FIGURE 1: Upper row: MRI obtained using bSSFP in sagittal (a), coronal (b), and axial (c) planes. Negative contrast in the center of the flank tumor suggests SPIO uptake or necrosis. Bottom row: 18F-FDG PET/MRI overlay in sagittal (d), axial (e), and coronal (f) planes using PET data obtained simultaneously with the MR data. A necrotic core is confirmed by low 18F-FDG activity aligned with the region of negative MR contrast.

IMAGING METHODS

- SPIO nanoparticles have high magnetic susceptibility. They locally alter the magnetic field in the MRI scanner. The R_2*(s^{-1}) relaxation rates of protons near SPIO are increased.
- All MRIs are obtained on a 3T pre-clinical Agilent (Menlo Park, CA, USA) scanner:
- Balanced steady state free precession (bSSFP) is performed first for high-resolution anatomical images (*TR/TE = 8/4 ms, α = 30°, 4 phase cycles, 4 averages, 256 x 170 x 170, FOV=38.4 mm x 25.5 mm x 25.5 mm, t=64min*)
  - high signal to noise ratio (SNR)
  - high resolution
  - sensitive to SPIO effects[3]
- TurboSPI is performed second (*TR/$TE_{effective}$= 250/10 ms, ETL=8, ESP=10ms, 96 x 96 x 48, FOV=30 mm x 30 mm x 30 mm, t=28min*)
  - quantification capabilities
  - higher SPIO specificity
- TurboSPI is a multi-echo single point imaging (SPI) sequence that is accelerated through compressed sensing techniques. R_2* maps are calculated from TurboSPI data[4].
- PET data (Fig. 1) was obtained for several mice, using the NuPET (Cubresa, Winnipeg, MB, CA) insert for simultaneous PET/MR.

GENERAL METHODS

- C57BL/6 mice are implanted with 5x10^5 C3 cells in 100 ul
- CD8+ cells are isolated from disease matched GFP mice & cultured *in vitro* for 7 days
- Cultured cells are loaded with ultra-small SPIO by passive incubation for 24 hours
- Cells are delivered via tail vein injection 24 hours are given for initial cell migration
- Mice are imaged according to the Imaging Methods protocol

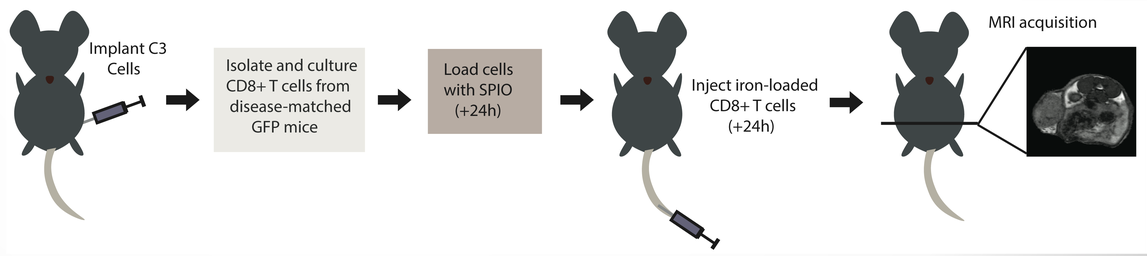

FIGURE 2: A summary schematic of the general method showing the steps from tumor implantation to imaging

RESULTS & DISCUSSION

IMAGE ANALYSIS

- After data acquisition, the under-sampled TurboSPI images were reconstructed using a supplemental guide image acquired immediately before the TurboSPI scan
- R_2* relaxation rate maps were calculated from the reconstructed multi-echo TurboSPI data. These maps are aligned with the axial slices of the bSSFP image and the set are analyzed in tandem.
- Figure 3 shows an example image set using this technique. The R_2* map in b) shows R_2* value by pixel intensity. It is not an iron specific metric, but analyzing R_2* value combined with the signal time course gives us information on iron content in regions of interest.
- The time course of the signal in c) shows a voxel with suspected SPIO-labeled cells, d) shows a periodic time course from a fat voxel, e) shows a time course indicating the presence of both fat and iron from a voxel in the fat pad anterior to the tumor.
- Interestingly, labeled CD8+ T cells seem to be primarily located at the tumor periphery, not in the central regions

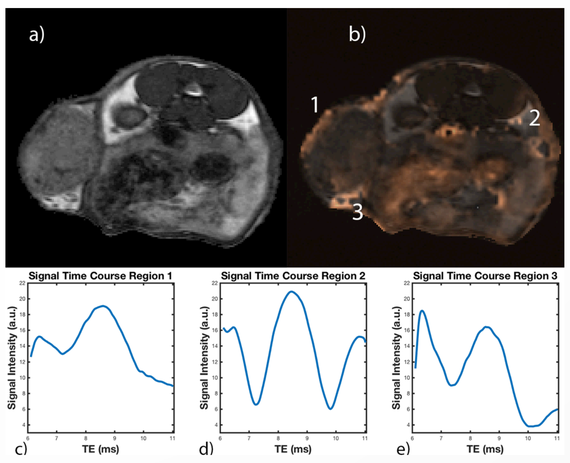

FIGURE 3: Upper row: a) is the bSSFP MRI reconstructed in the axial plane, b) is an R_2* map (pixel intensity in s^{-1}) overlaid on the bSSFP slice that shows a distribution of R_2* in the same plane. Region 1 indicates a pixel with a significant amount of iron in the tumor, Region 2 indicates signal from pure fat (oscillations are fat specific) and 3 suggests a combination of iron and fat. Bottom row: the MR signal time course for region 1 (c), region 2 (d), and region 3 (e)

QUANTIFICATION

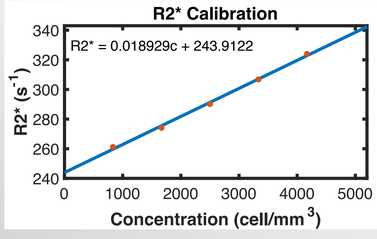

FIGURE 4: R_2* calibration for different concentrations of SPIO loaded cells.

- R_2* calibration was performed for cellular quantification purposes & Samples were prepared with known cell concentrations and the bulk R_2* was calculated using line width
- Cellular concentrations are calculated from the voxel intensities in the R_2* map and can be converted to numbers of cells/voxel using the voxel size
- For example, the voxel indicated by region 1 in Figure 3 has approximately 540 cells/voxel

VERIFICATION

- Immunohistochemistry was performed on the excised tumor to confirm the existence of injected (SPIO labeled) cells. Cells were labeled with DAPI (nuclear stain), and iron label has Rhodamine B attached.

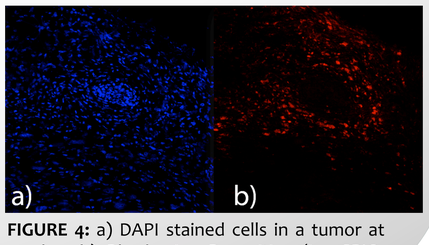

FIGURE 4: a) DAPI stained cells in a tumor at week 4 b) Rhodamine B positive (i.e. SPIO-labeled) cells in the sectioned tumor

CONCLUSION

- The R_2* mapping that is performed using TurboSPI offers more information than the bSSFP images. The specificity of the TurboSPI analysis results in more "true positive" regions and therefore more accurate evaluation of cellular recruitment. Furthermore, the R_2* mapping and calibration provides superior cell quantification to previous techniques. We are currently evaluating the quantitative results of CD8+ T cell recruitment in a tumor model
- We suggest that the imaging protocol for SPIO labeled cell tracking is most powerful when the two sequences, bSSFP and TurboSPI are used together, incorporating the strengths of each technique: the specificity and quantification from TurboSPI, and the resolution and SNR of bSSFP.
- Parallel work is currently being performed using these cell tracking techniques to investigate the cellular response to different immunotherapy treatments (see poster by Tremblay et al.). CD8+ T cells and dendritic cells are being studied, particularly their response to immunotherapy, specifically a peptide-based vaccine, a checkpoint inhibitor and a combination of both therapies.

1. Brewer KD et al. Clearance of depot vaccine SPIO-labeled antigen and substrate visualized using MRI. *Vaccine* 2014, **32**:6956-6. 2. Rioux JA et al. Quantification of superparamagnetic iron oxide with large dynamic range using TurboSPI. *Journal of Magnetic Resonance* 2012, **216**:152-60. 3. Majumdar S. et al. The influence of pulse sequence on the relaxation effects of superparamagnetic iron oxide contrast agents. *Magnetic Resonance in Medicine*, 1989, **10**2:289-301. 4. Rioux JA et al. 3D single point imaging with compressed sensing provides high temporal resolution R2* mapping for in vivo preclinical application. *MAGMA* 2016; epub ahead of print.

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